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Influence of Placentation on High Density Lipoproteins in Adult Males: The NHLBI Twin Study

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Abstract. Dizygotic (DZ) World War II veteran twins who participated in the National Heart Lung and Blood Institute (NHLBI) Twin Study have been reported to have greater variance than monozygotic (MZ) twins for plasma high-density lipoprotein cholesterol (HDL-C), cholesterol in the low-density fraction of HDL (HDL_2-C) and apolipoprotein A-I, a major protein component of HDL. It was hypothesized that a possible source of this difference in zygosity variance could be prenatal environmental influences related to placental type. Dermatoglyphics were used to provide a retrospective index of placental type in a subset of the NHLBI MZ twins aged 59-70. The MZ twins classified as dichorionic were found to have significantly greater within-pair variability than the monochorionic MZ twins for HDL-C, HDL_2-C and Apo A-I. These findings indicate that intrauterine environmental influences on HDL are manifest later in life.

Key words: Cholesterol, Dermatoglyphics, High-density lipoprotein, Twin placentation

INTRODUCTION

Over the three examinations of the National Heart, Lung and Blood Institute (NHLBI) twin study in 1969-73, 1981-82 and 1986-87, the fraternal (DZ) twins have developed a progressively greater total variance than identical (MZ) twins for high-density lipoprotein cholesterol (HDL-C) [10]. At the third examination, when the twins ranged in age from 59 to 70, HDL fractions (HDL_2, density = 1.063 to 1.125 g/ml and HDL_3, density = 1.125 to 1.210) and apolipoprotein A-I (Apo A-I) were measured. The variance of Apo A-I was also found to be significantly greater in the DZ than the MZ twins [19]

and most of the greater DZ variance in HDL-C could be accounted for by variance of the HDL_2-C fraction [10].

Greater DZ than MZ variance could be due to different environmental or genetic influences on the two zygosities [10]. Christian and Kang [11] estimated that 20% of the variance of total plasma cholesterol was due to maternal effects and postulated that these maternal effects might be due to the prenatal environment provided by the mother. Corey et al [12] found that the variance of total plasma cholesterol in cord blood, which has a higher percentage of HDL-C than in adults, was significantly greater in dichorionic MZ twins than in the cord blood of monochorionic MZ twins. Chen et al [9] reported slightly higher, but nonsignificant within-pair differences in cord blood HDL-C and total cholesterol in dichorionic vs monochorionic MZ twins. Greater variance of dichorionic than monochorionic twins could account for the greater DZ than MZ variance in the NHLBI twins because all DZ twins are dichorionic and only one-third of MZ twins are dichorionic [8]. In this publication, we report that HDL-C, HDL_2-C and Apo A-I, measured when the NHLBI twins were 59-70 years old, were significantly more variable within dichorionic than monochorionic MZ twins, with placental type determined retrospectively using a dermatoglyphic index.

MATERIALS AND METHODS

The NHLBI twin stud is a longitudinal study of white, male veteran twin pairs born between 1917 and 1927 [13,14]. At the third examination in 1986-87, 622 members of the cohort were examined including 134 MZ pairs. Lipid fractions were measured by the Stanford Center for Research in Disease Prevention Biochemistry Laboratory under the direction of Peter D. Wood and included total cholesterol (Total-C) using the method of Allain et al [1] and HDL-C by the method of Warnick et al [26]. The HDL_2 subfraction was precipitated, HDL_3-C measured, and HDL_2-C obtained by subtraction using the methods of Warnick et al [27]. Low-density lipoprotein cholesterol was estimated by the equation of Friedewald et al [15] when triglycerides were less than 300 mg/dl [LDL-C = Total-C − (HDL-C + triglycerides/5)]. Triglycerides were measured by the method of Sampson et al [25]. Plasma Apo A-I and B were measured at the Lipid Metabolism Laboratory at Tufts University under the direction of Dr. Ernest J. Schaefer using the method of Ordovas et al [22].

Placental type was retrospectively assessed in the MZ twins in the NHLBI twin study using a discriminant function derived from dermatoglyphic (finger, palm and footprint) variables collected in 241 pairs of MZ twins from other cohorts in which the placenta type was established at birth [23]. Conservative cutoffs of the placental type index, which minimized the overlap in scores beteween known monochorionic and known dichorionic MZ twins and correctly classified 92.5% of such pairs having scores falling in the tails of the distribution, were used to assign NHLBI MZ twin-pairs into monochorionic or dichorionic groups [24]. Of the 134 MZ pairs participating at exam three, 80-90 pairs had data for each of the lipid variables and the placental type index. For the individual lipid variables, there were 19-24 pairs of twins in the "monochorionic tail" and 13-16 in the "dichorionic tail".

The F test (within dichorionic mean square/within monochorionic mean square) was

used to test the hypothesis that the dichorionic twins had greater within-pair variability than the monochorionic twins. Regression and correlation analyses were used to measure the relationships of the lipid variables with the dermatoglyphic index, using all of the twins with complete dermatoglyphic data. The SAS statistical package (SAS Institute, Cary, North Carolina) was used.

RESULTS

Table 1 shows the within-pair mean squares of the lipids measured at examination three for the subset of twins classified as monochorionic or dichorionic by being in the tails of the distribution of the dermatoglyphic index of placentation. The within-pair mean square of the dichorionic MZ pairs was significantly ($P < 0.05$) greater than the within-pair mean square of monochorionic MZ pairs for HDL-C, HDL_2-C, and Apo A-I, but not for HDL_3-C, Apo B, LDL-C or Total Cholesterol. Comparison of the distribution of within-pair differences for the full sample indicated that a single dichorionic MZ pair was an outlier for HDL-C and HDL_2-C using either the test of the standardized deviate or the test of the skewness coefficient [5]. To determine if the differences observed between monochorionic and dichorionic MZ twins for HDL-C and HDL_2-C in Table 1 were principally the result of this single outlier pair, two approaches were utilized. First, a logarithm of the within-pair differences removed the outlier status of this pair. For HDL-C, the log transformed data resulted in a change in probability for the difference in within-pair mean squares from 0.005 to 0.08. The probability for log transformed HDL_2-C remained significant ($P < 0.01$). Removal of the outlier pair from the analysis of untransformed data

Table 1 - Within-pair mean squares of lipids at exam 3 in monochorionic and dichorionic MZ twins and MZ twins in the full sample

Variable	Full sample (a)		(b)		(c)		F (b/c)	P-value
	WMZ[a]	(n)	WDCMZ[a]	(n)	WMCMZ[a]	(n)		
HDL-C	47.4	(124)	88.6*	(16)	27.5	(24)	3.22	0.005
HDL_2-C	18.4	(124)	37.9*	(16)	8.1*	(24)	4.69	0.0004
HDL_3-C	16.1	(124)	19.0	(16)	12.2	(24)	1.55	0.16
Apo A-I	334	(109)	612*	(13)	269	(19)	2.28	0.05
Apo B	281	(109)	204	(13)	240	(19)	0.85	0.61
LDL-C	439	(115)	226	(14)	425	(22)	0.53	0.89
Total cholesterol	660	(124)	607	(16)	473	(24)	1.28	0.28

* = $P < 0.05$ vs full sample; all P-values are one-tailed.

[a] WMZ, within-pair mean square of all MZ twins seen at exam 3; WDCMZ and WMCMZ, within-pair mean squares for dichorionic and monochorionic twin pairs, respectively; number of twin pairs are in parentheses.

led to similar results as with log transformation. The differences observed in Table 1 for HDL-C and HDL_2-C were not completely accounted for by the outlier.

The correlation coefficient of the within-pair differences of HDL-C, HDL_2-C and Apo A-I for the full sample of twins and the placentation index score, in which a positive value indicated a monochorionic pair, was –0.15 ($P = 0.08$), –0.20 ($P = 0.03$) and 0.21 ($P = 0.03$), respectively. When HDL_2-C difference was entered in the first step of a multiple stepwise regression analysis, with the placentation index score as the dependent variable, none of the other lipid components measured at examination three made a significant additional contribution to the multiple regression.

To determine if the association between placental type index and cholesterol was present at the first and second exams of the NHLBI twin study, the HDL-C within-pair mean squares were compared for the twins judged most likely to be monochorionic and dichorionic for the three examinations (Table 2). The total cohort of twins available to each examination was also compared to the cohort of twins that returned to the third examination (returnees). For both cohorts, the monochorionic mean squares were slightly larger than the dichorionic within-pair mean squares at exam one. However, these differences were well within what could be expected due to sampling variation. At exam two, the dichorionic mean squares were larger than the monochorionic mean squares for both the total cohort and the returnees, but not significantly so. Only at the third examination did the dichorionic twins have significantly greater within-pair variation than the monochorionic pairs. Correlation analysis using all of the twins with dermatoglyphic indices revealed a similar pattern of no significant associations of HDL-C and the dermatoglyphic index until exam three. These data suggest that the association of HDL-C with placental type is becoming stronger as the twins age.

Table 2 - Comparison of the within-pair mean squares for HDL-C at the three examinations of twins judged monochorionic and dichorionic using a dermatoglyphic index of placentation

	(a) WDCMZ[a]	(b) WMCMZ	(a/b) P-value
Exam One			
Total Cohort	48.6 (23)	55.7 (33)	0.63
Returnees	49.2 (16)	71.5 (23)	0.78
Exam Two			
Total Cohort	78.1 (22)	69.3 (31)	0.37
Returnees	91.2 (15)	61.9 (22)	0.20
Exam Three			
Returnees	88.6 (16)	27.5 (24)	0.005

[a] WDCMZ and WMCMZ, within-pair mean squares for dichorionic and monochorionic twin pairs respectively; number of twin pairs is in parentheses.

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DISCUSSION

HDL-C, HDL_2-C and Apo A-I all have been reported to be inversely associated with the risk of coronary heart disease [2,3,7,16-18,20,21]. The correlation of HDL-C was 0.80 with both HDL_2-C and Apo A-I for MZ pairs at exam three and the correlation of Apo A-I and HDL_2-C was 0.59. The finding that the variation of HDL-C, HDL_2-C and Apo A-I within twin-pairs is larger in dichorionic than monochorionic MZ twins must be due to environmental influences as both types of MZ cotwins are genetically identical. Because all DZ twins are dichorionic and only about one-third of MZ twins are dichorionic, the greater DZ variance of HDL-C, HDL_2-C [10] and Apo A-I [19] in this same cohort could in part be due to the prenatal environment associated with placental type.

The association of placental type and the dermatoglyphic index and the greater DZ than MZ variance for HDL-C appeared to increase as the twins aged. Christian et al [10] reported that the total variance of the DZ twins was 28% greater than the MZ twins at exam one ($P < 0.05$), 37% greater at exam two ($P < 0.01$) and 56% greater at exam three ($P < 0.01$). The finding of evidence for prenatal effects on an important coronary heart disease risk factor supports the hypothesis of Barker [4] who suggested that differences in individual susceptibility to coronary heart disease, often attributed to genetic causes because no environmental covariates were found, may be due in part to the intrauterine environment.

While it is rewarding to find data that fit an *a priori* hypothesis, it is critical to test the hypothesis of twins of different ages, female twins and twins of known placental type. It could also be rewarding to serach for specific prenatal environmental conditions which influence HDL_2-C and Apo A-I values in singletons. For example, Bodurtha et al [6] reported lower levels of HDL_2-C in adolescent twins of smoking mothers compared with twins of nonsmoking mothers. If our findings are confirmed, it could lead to methods of influencing cholesterol levels ans the risk for coronary heart disease by changing the prenatal environment.

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